

Toxicology of the Fluoroalkenes: Review and Research Needs

by J. Wesley Clayton*

In this review of the published literature on the toxicology of fluoroalkenes several features emerge and research needs are evident. The fluoroalkenes vary widely in acute inhalation toxicity. Those, such as perfluoroisobutylene, PFIB, the most highly toxic member, attacks the pulmonary epithelium of rats eventuating in edema and death after a delay of about one day. Other fluoroalkenes, such as hexafluoropropylene (HFP) or chlorotrifluoroethylene (CTFE), also cause pulmonary injury but at lower concentrations produce concentration dependent changes in the renal concentrating mechanism of the rat. Changes in the CNS of rats and rabbits have also been reported for CTFE. CTFE, in repeated exposures, has produced blood pressure changes in dogs, CNS effects and changes in the erythropoietic system. This variety of responses indicates the need for investigation. Chronic effects have not been sufficiently studied for PFIB and HFP. Thus pointing up the desirability for study. Mechanisms of action research for fluoroalkenes is an important area of need. While several ideas have been suggested, there are no data to support them. The nucleophilic sensitivity of the fluoroalkenes and the potential carcinogenic effects stemming therefrom suggests a need field for investigation. We also can readily perceive the needs for the evaluation of effects on reproduction (including mutagenesis and teratogenesis), metabolism pulmonary functions, cellular function and structure. Epidemiologic studies on occupationally exposed populations are desirable in order to adequately define human health hazard from these fluorocarbons.

Introduction

Because of legitimate concern about the toxicologic and environmental effects of widely used fluoroalkane propellants such as dichlorodifluoromethane (fluorocarbon 12), trichlorofluoromethane (fluorocarbon 11) and others which have been in commercial use for decades, other fluorocarbons such as the fluoroalkenes, have only been cursorily evaluated for toxic and other hazards. It is the purpose of this paper to review the relevant published literature on the toxicology of several fluoroalkenes and to define areas of need for future toxicologic research.

That fluoroalkenes are important in toxicologic evaluation, has been pointed out by Krespan (*1*). He points out that it was recognized more than 40 years ago that fluoroalkenes such as tetrafluoroethylene and chlorotrifluoroethylene could be polymerized by free-radical catalysis. This development led naturally to a preparation of several kinds of fluoropolymers formed from the variety of fluoroalkenes which were subsequently synthe-

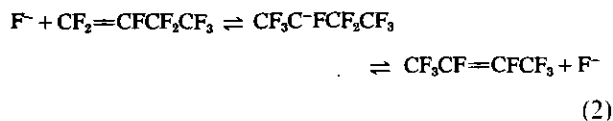
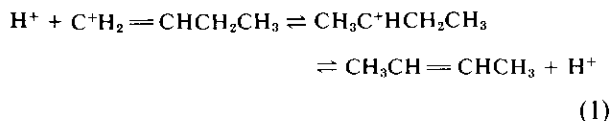
sized. Thus, fluoroalkenes, insofar as health hazards were concerned, were treated mainly as industrial chemicals, prepared by the manufacturer, subjected to appropriate toxicity evaluation, and handled with the caution considered their due. Consequently, they rarely if ever came in contact with the public as the fluoroalkanes have. However, isolated accidental exposures in industry brought the toxic potential of the fluoroalkenes dramatically to our attention. It would seem that the industrial controls requisite to the safe manufacture and handling of these chemicals precluded significant health hazards. Accordingly, little toxicologic research beyond single and shortterm repeated inhalation exposures has been available in the published literature.

Chemistry

Fluoroalkenes differ from their hydrocarbon counterparts in that the area of the double bond tends not to be rich, but deficient in electrons because of the strong electronegativity of the fluorine atoms attached to adjacent carbons. Thus fluoroalkenes are subject to nucleophilic attack and are prey for bases or other nucleophiles, e.g., fluoride,

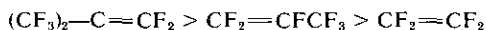
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OH or NH₂. Krespan (1) shows how the fluoride ion (F⁻) is related to the chemistry of the fluoroalkenes in the same way as the proton (H⁺) is related to the alkene hydrocarbons:



In reaction (2), migration of the double bond is accomplished by the movement of the nucleophile F⁻ into the double-bond electrophilic center with subsequent leaving of F⁻ to proceed down the chain. The same event for the alkene hydrocarbons is shown in Eq. (1). In alkene hydrocarbons, the area of the double bond is electron rich thus attracting an electrophilic proton.

Where a locus of the type present in the double bond area of the fluoroalkenes exerts a major influence on its electronic properties, it would seem likely that biological activity would be largely influenced by the area of low electron density between adjacent carbons and the consequent susceptibility to nucleophilic attack. According to Chambers and Mobbs (2) and Cook and Pierce (3), susceptibility of fluoroalkenes to nucleophilic attack is in the following order:



The inhalation toxicity is in the same order perfluoroisobutylene > hexafluoropropylene > tetrafluoroethylene (Table 1). However, experimental data bearing on this potential relationship are lacking, as is information on the biologic or toxicologic mechanisms of action among the fluoroalkenes. For example, Danishevskii and Kochanov (4) claim that the high toxicity of perfluoroisobutylene (PFIB) stems from *in vivo* carbonyl fluoride formation. Against this view, however, COF₂ is lower in acute inhalation toxicity than PFIB (5), and it produces different biologic sequelae. The picture of COF₂ toxicity is that of hydrogen fluoride. The approximate lethal concentration (ALC) of COF₂ (4-hr exposures in rats) is 100 ppm, which would correspond to 200 ppm of HF, a concentration in the lethal range. It is only conjecture, but the susceptibility of PFIB to nucleophilic attack might constitute the basis for its high toxicity.

Acute Inhalation Toxicity

Summaries of acute inhalation toxicity of several fluoroalkenes are found in Tables 1 and 2. A wide range of toxicity is readily apparent in Table 1, and while it may seem that toxicity in this class is inversely related to the number of fluorine atoms, inadequate knowledge of toxic mechanisms at present obviates any such conclusion. It is more likely that some biological action related to the double bond, as postulated above, is predominantly responsible for the high toxicity of PFIB, rather than its complement of eight fluorine atoms. The toxic mechanism however remains to be demonstrated.

Table 1. Inhalation toxicity of several fluoroalkenes.^a

Structure	No. F atoms	Acute toxicity for rats ^b	
		ALC, ppm	LC ₅₀ ppm
CH ₂ =CHF	1	>800,000 ^b	—
CF ₂ =CH ₂	2	128,000	—
		>800,000 ^c	—
CF ₂ =CF ₂	4	—	40,000
CF ₃ CF=CF ₂	6	—	3,000
(CF ₃) ₂ C=CF ₂	8	0.5, 0.76 ^d	—

^aData of Clayton (5).

^b4-hr exposures except where noted.

^c80% CH₂=CHF, 20% O₂; 12.5-hr exposure.

^d80% CH₂=CF₂, 20% O₂; 19-hr exposure.

^e0.5 ppm exposure was 6 hr; the 0.76 ppm exposure was 4 hr.

Table 2. Inhalation toxicity of several halogenated alkenes.^a

Structure	No. F atoms	No. Cl atoms	Acute toxicity for rats ^b	
			ALC, ppm	LC ₅₀ ppm
CCl ₂ =CH ₂	0	2	32,000	—
CHCl=CCl ₂	0	3	8,000	—
CCl ₂ =CCl ₂	0	4	4,000	—
CCl ₂ =CF ₂	2	2	1,000	—
CClF=CF ₂	3	1	—	1000

^aData of Clayton (5).

^bAll 4-hr exposures.

Generally, fluorinated alkenes are less toxic than chlorinated alkenes. Table 3 brings together three homologous pairs illustrating this point. A direct relationship between toxicity and number of chlorine atoms is illustrated by the sequence signifying acute inhalation toxicity of these chlorinated alkenes as judged by animal inhalation exposures. In order of decreasing toxicity we have:



Illustrative of fluoroalkenes of relatively low toxicity are vinyl fluoride (VF) (CH₂=CHF) and vinylidene fluoride (VF₂) (CH₂=CF₂). As indicated

Table 3. Toxicity comparisons among some halogenated alkenes (rats).^a

Structure	Acute inhalation toxicity, ALC, ppm (by volume) ^b
CH ₂ =CHCl	>250,000 ^c
CH ₂ =CHF	>800,000 ^d
CH ₂ =CCl ₂	32,000
	128,000
CH ₂ =CF ₂	>800,000 ^e
CCl ₂ =CCl ₂	4,000
CF ₂ =CF ₂	40,000

^aData of Clayton (5).

^bAll 4-hr exposures except where noted.

^cGuinea pigs, 8-hr exposure.

^d80% CH₂=CHF, 20% O₂; 12.5 hr exposure.

^e80% CH₂=CF₂, 20% O₂; 19-hr exposure.

by Table 3, high concentrations were not lethal for rats exposed to either VF or VF2 for 12.5 and 19 hr, respectively. In the work by Lester and Greenberg (6) there was no organ damage reported. Limperos (7) conducted repeated exposures with male and female rats inhaling 100,000 ppm VF 7 hr/day, 5 days/week, for 30 exposures. There were no fatalities; rats gained weight normally, showed no obvious behavioral changes, or tissue damage, as evaluated by microscopic examination.

Fluoroalkenes of moderate to slight toxicity may be exemplified by tetrafluoroethylene (TFE), hexafluoropropylene (HFP), and chlorotrifluoroethylene (CTFE). These compounds are irritating to the respiratory tract and lungs in lethal concentrations as judged by animal exposures, but in addition they also can cause kidney injury (8-10). Single exposures of rats to varying concentrations of CTFE have produced evidence of kidney dysfunction. Radford (11), as reported by Zapp (8) demonstrated that laboratory rats ingesting a dry diet *ad libitum* voluntarily limited their water intake and consequently excreted a maximally concentrated urine. Tracking the water intake, urine volume, and solute concentration of several rats, Radford found a high degree of uniformity in the response of individual rats for these variables. This suggested an exquisite response system relating to the concentrating mechanism of the renal tubular cells in the rat. When this mechanism was disturbed, water intake and urine volume increased while solute concentration decreased. In order to determine possible "dose" relationships, Radford exposed groups of male rats for 4 hr in an inhalation chamber to 125, 240, 340, or 460 ppm of chlorotrifluoroethylene (CTFE). Work reported by Hood et al. (10) had disclosed kidney injury in rats inhaling CTFE. In Radford's work, half of the rats inhaling 460 ppm died within one week after exposure. The remaining animals survived and were observed for signs of renal dysfunc-

tion for 39 days. Figure 1 depicts the effects of the various levels of CTFE on the rat's ability to concentrate its urine. Of the three variables shown, body weight was the least sensitive to CTFE. Rats inhaling 460 ppm showed weight depression as compared to the pre-exposure values. At 340, 240, and 125 ppm, it is debatable whether or not the body weight curves show a weight depression related to CTFE exposure because food consumption was also down slightly. Variables reflecting renal function were the most sensitive indices of effects. All treatment levels exerted an effect on the renal concentrating mechanism, and this effect varied in a dose-related fashion.

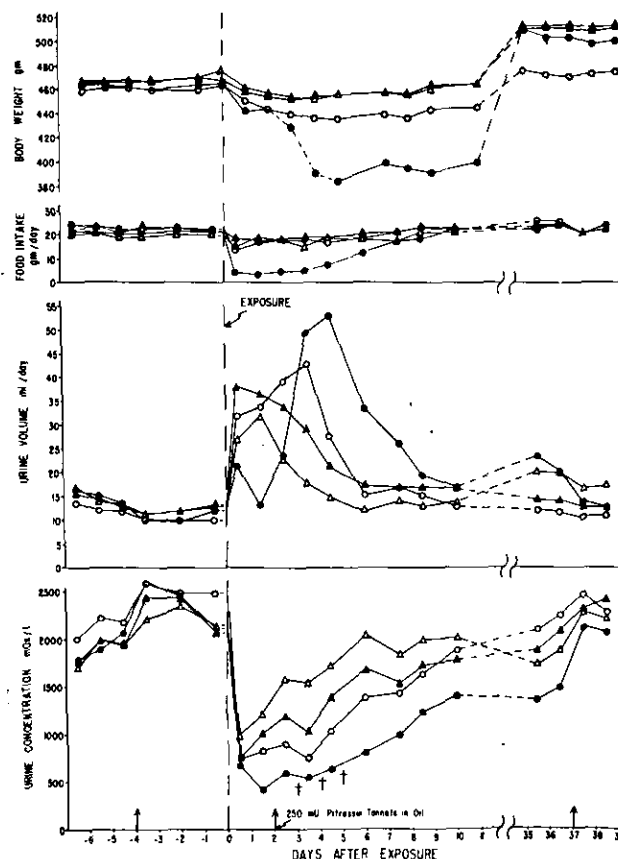


FIGURE 1. Effects of single exposure of chlorotrifluoroethylene: (○) 125 ppm; (▲) 240 ppm; (○) 340 ppm; (●) 460 ppm. Mean values for groups of six male rats.

Figure 2 illustrates the dose dose/concentration relationships derived from the foregoing CTFE exposures and several doses administered by subcutaneous injections of mercuric chloride, a comparative control. Functional impairment of the kidney can therefore be expressed as a decline of urine solute concentration and increased water intake with in-

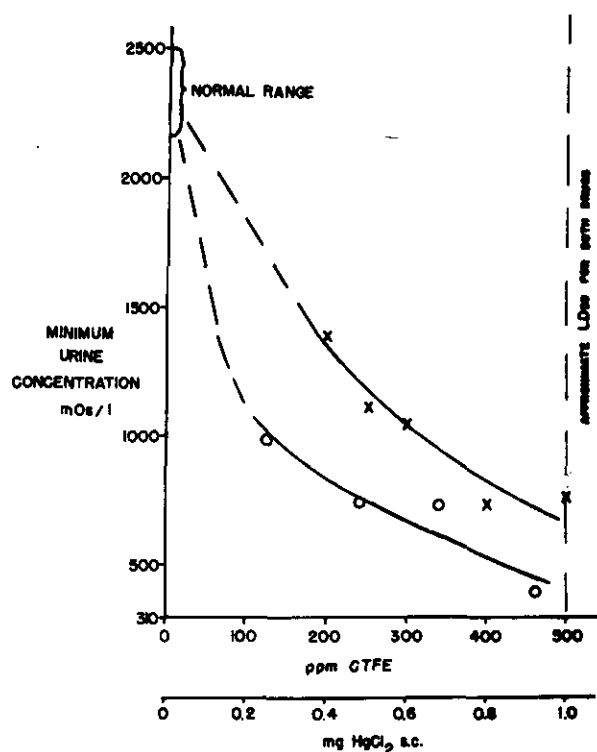


FIGURE 2. Effects of (x) HgCl_2 and (o) CTFE on rat kidneys.

creasing CTFE concentrations. The threshold concentration for this effect appears to be around 100 ppm, approximately one tenth the 4-hr, rat LC_{50} of 1000 ppm (5). Hexafluoropropylene (HFP) and tetrafluoroethylene (TFE) provoked similar responses in the rat kidney, but these were both less active in this regard than CTFE. For HFP the 4-hr rat LC_{50} was found to be 3000 ppm, and the threshold concentration for impairment of the rat renal concentrating function was approximately 400 ppm (Fig. 3) about one tenth the LC_{50} , as with CTFE. For TFE these values were 40,000 ppm and 500 ppm respectively (5). These findings in rats suggests a physiological basis for setting workplace standards for the fluoroalkenes, but it is clear that detailed studies, say on isolated renal tubules and electron microscopy, are needed to elucidate the finer aspects of the phenomenon just described. Furthermore, other fluoroalkenes, e.g., VF, VF₂, dichlorohexafluorobutene, hexafluorocyclobutene, and perfluoroisobutene need to be subjected to evaluation of effects on renal function. In addition, long-term, repeated, low level exposures are needed.

Microscopic examination of tissues from male rats exposed to 800, 900, 1000, or 1200 ppm of CTFE for 4 hr was reported by Hood et al. (10). The LC_{50} was calculated as 1000 ppm. Death occurred 1–11 days after the inhalation exposure. Pathology revealed pulmonary edema, pleural effusion and degeneration

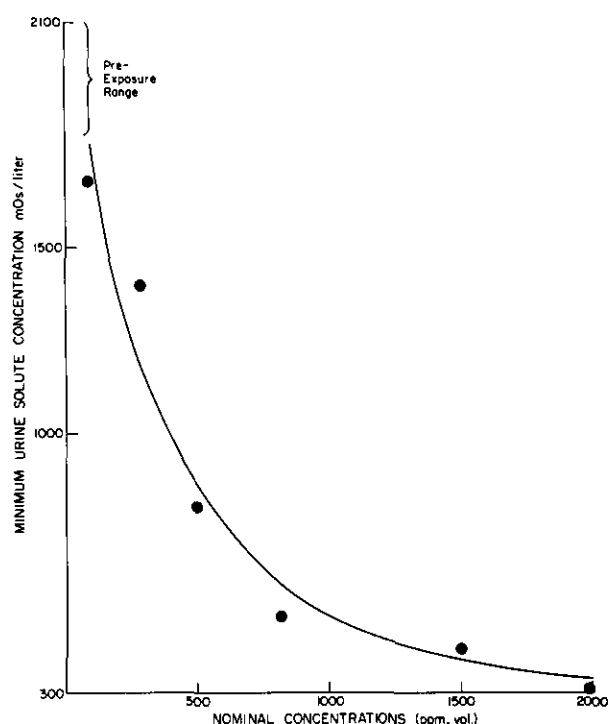


FIGURE 3. Effects of hexafluoropropylene on rat renal concentration for 4 hr exposure.

of the renal tubules. Clayton conducted kidney function studies on these rats and showed that sublethal concentrations caused increased urine volume which reached a high point 2 to 4 days after exposure and gradually returned to lower volumes in the 14-day observation period following exposure. These findings agree with those observed by Radford. We need now to study this post-exposure phase in more detail. It would be important to follow the anatomical changes (light and electron microscopy) occurring at several points along the curves plotted in Figure 2 describing renal function changes. Of particular significance is the so-called recovery phase. Is it in fact "recovery?" What is the significance of these events for humans accidentally exposed to these fluoroalkenes?

Kochanov (12) exposed groups of rats and rabbits for 2 hr each to various concentrations of CTFE. The LC_{50} for both rats and rabbits was determined as 5,040 ppm. The concentrations resulting in death of all animals from the 2-hr exposure were 5544 ppm and 7560 ppm for rabbits and rats, respectively. The animals became first excited, apathetic, then dyspneic and inactive during exposure. There was also impairment of coordination at the highest concentration of approximately 10,000 ppm. Deaths occurred during the first few days after exposure. Histology of animals that died disclosed congestion of internal organs, changes in the brain, and necrosis of

the kidney tubules. The authors did not report on the histology of animals that survived exposure. Neither did they describe any changes in the water intake or volume of urine excreted. It is also notable that the LCT_{50} of 10,000 ppm-hours reported by Kochanov (12) is about 2.5 times that of 4000 ppm-hours determined by Hood et al. (10). This raises the question of sample identity. Neither of the two groups reported on the composition of their samples or the contaminants.

Paulet and Desbrousses (13) exposed Swiss mice and Wistar rats to hexafluoropropylene for time periods of 0.5-8 hr. The results of the experiments of these authors are summarized in Table 4. The lowest lethal concentrations for the 8-hr exposures were 400 ppm for mice and 2000 ppm for rats. Deaths occurred during the succeeding 10 days following exposures. At the high levels deaths occurred within one day.

Table 4. Acute inhalation toxicity of hexafluoropropylene.^a

Duration of exposure, hr	LC ₅₀ , ppm	
	Mice	Rats
1/2	3000	15,750
2	1200	4,000
4	750	2,800
6	680	2,350
8	600	2,400

^aData of Paulet and Desbrousses (13).

Danishevskii and Kochanov (4) report studies on rats exposed to hexafluoropropylene, tetrafluoroethylene and chlorotrifluoroethylene. The following lethal levels were reported for 2-hr exposures: hexafluoropropylene, 3,240-13,365 ppm; tetrafluoroethylene, 25,000 ppm; chlorotrifluoroethylene, 7,560 ppm. Histology of rats which died after exposure to these three fluoroalkenes showed pulmonary congestion and edema with degenerative changes in liver and kidney. These levels would be expected to be lethal on the grounds of previously described studies. Changes that might have occurred in urine volume and water intake were not reported.

Investigating the toxicity of tetrafluoroethylene, Zhermerdei (14) exposed rats and rabbits for 2 hr each to concentrations in the range of 5000 to 100,000 ppm by volume. The lowest lethal concentration for the rats exposed was 25,000 ppm. The time of death of the rats was in direct proportion to the concentration and varied from 3 hr after exposure to 16 days and more. Rats exposed to 5000 ppm showed no signs of toxicity. At higher concentrations, there was inactivity, rapid respiration and somnolence. After exposure ended, there was depression, slow respiration, inactivity and occasional

tetanic convulsions elicited by external stimuli. For rabbits, the lowest lethal concentration was 40,000 ppm. At 10,000 ppm there were no signs of toxicity; above 10,000 ppm, there was inactivity and a decrease in respiration rate. After exposure, the rabbits appeared apathetic, lost appetite, and the rate of respiration increased. Rabbits which succumbed experienced a convulsion before death. Pathology of rats and rabbits disclosed congestion of organs, especially the brain, hemorrhage of the lungs and spleen, and degenerative changes in the kidneys. Considering probable differences in the kind and quantity of contaminants in the various samples, the work reported by Danishevskii and Kochanov (4) and Zhermerdei (14) reveals the same order of toxicity for the several fluoroalkenes as reported earlier.

Referring to Table I, perfluoroisobutylene (PFIB) is by far the most toxic fluoroalkene known. Clayton (15) reported the acute inhalation of PFIB. Table 5 summarizes the data from the exposures, and Figure 4 discloses time/concentration relationships with mortality. Rats succumbing to PFIB showed progressively severe tachypnea and cyanosis and died as a result of pulmonary edema with no effects discernible in other organ systems. From these data and similar animal experiments with phosgene, PFIB ($LCT_{50} = 3$ ppm-hr) is about ten times as toxic as phosgene ($LCT_{50} = 25$ to 40 ppm-hr). The acute toxic action of these two irritants appears to be directed solely at the lung. Longer-term exposures to PFIB at levels without deleterious effects on the lung could exert other actions—possibly on the kidney, as has been reported for CTFE and HFP.

Exposures of rats and mice to PFIB are reported by Danishevskii and Kochanov (4). In 2-hr exposures all rats succumbed to a concentration of 1.8 ppm (vol) (3.7 ppm-hr), and the maximum tolerated concentration was 1.6 ppm. Mice tolerated a concentration of 0.6 ppm while 1.2 ppm (2.4 ppm-hr) and 1.8 ppm (3.7 ppm-hr) were the minimum lethal and absolute lethal concentrations, respectively. Histology disclosed pulmonary hemorrhages and edema and degenerative changes in the kidney, viz., albuminous, granular dystrophy and plasmolysis [sic] of the cells of tubules. Degenerative changes, sometimes fatty, in the liver were also reported. A relationship between the degree of the histologic reaction and the level of exposure is not delineated by these authors. This work is in agreement with that already summarized with respect to lung changes. Changes in other organs however are not consistent.

With the exception of Kochanov's work on CTFE (12), Russian investigations of the toxicity of

Table 5. Summary of single exposures of rats to perfluoroisobutylene.^a

Nominal concn, ppm (by vol.)	Time of exposure, hr	Mortality ratio, (no. dead/no. exposed)	Clinical signs	Pathological changes
1.0	4.25	2/2	Dyspnea, cyanosis, gasping convulsions	Acute pulmonary edema
0.5	5.33	2/2	Slight dyspnea after exposure. Both died overnight	Acute pulmonary edema
0.3	6.00	0/2	None during exposure. Slight increase in respiration rate and weight loss next day. Recovered thereafter	None observed 9 days after exposure

^aData of Haskell Laboratory, unpublished.

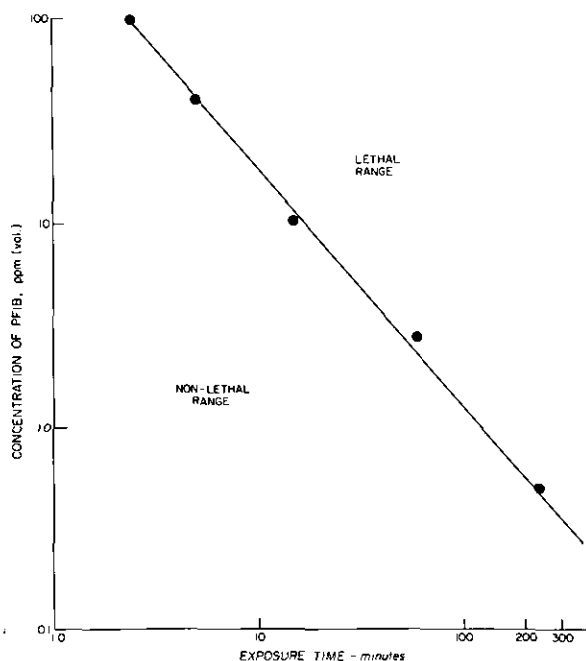


FIGURE 4. Rat mortality from inhaled perfluoroisobutene.

CTFE, TFE, HFP, and PFIB disclose the same order of toxicity that American authors report. However, the histologic changes appeared to differ somewhat; these need to be clarified.

In their studies on fluoride ion excretion by rats inhaling fluoroalkenes, Dilley et al. (16) found that hexafluoropropene (30 min exposure at 2600 ppm) produced increased urine excretion as well as increased fluoride excretion. The diuretic effect was most pronounced and lasted longer with HFP compared to the other fluorocarbons investigated. Rats inhaling HFP displayed histologic changes in the kidneys which were described as dilation and necrosis of the proximal tubules, cytoplasmic eosinophilia, and sloughing into the intraluminal areas. The authors conclude that the fluoroalkenes

or a metabolite thereof caused the increase in urine volumes observed. Because only one concentration was used, it is not possible to determine the threshold levels of these fluorocarbons. However, the work is in agreement with those previously cited which demonstrated renal toxicity for the fluoroalkenes studied.

Dilley et al. (16) also studied the effects of tetrafluoroethylene (TFE), trifluoroethylene, vinylidene fluoride, vinyl fluoride, and hexafluoroethane. Changes like those reported for HFP were observed, however, the extent of these were not as marked as those noted for HFP. It is likely that the changes would be dose-related and that experiments at additional levels would reveal this.

Repeated Inhalation Toxicity

Few studies of the effects of repeated animal exposures to fluoroalkenes have been reported, yet these fluorocarbons possess the greatest potential for biological effects.

Chlorotrifluoroethylene (CTFE), Repeated Exposures

After Hood et al. (10) had determined the LC_{50} for CTFE in rats, viz., 1000 ppm, these investigators went on to study the effects of repeated exposures, Phases I and II.

In Phase I, three dogs, male and female rats, male guinea pigs, and male rabbits were exposed to 300 ppm CTFE, 4 hr per day, 5 days per week. Eighteen exposures were actually conducted. Rats showed a slight weight loss but no other signs of toxic action. There were changes in the renal tubules. In the guinea pigs there was one death after the sixth exposure, loss of body weight, and normal histology. Rabbits showed two deaths after the fourth and fifth exposures, loss of body weight, normal

histology. In dogs, blood pressure and heart rate were normal; there was mild leucopenia and granulocytopenia during the second and third day of each week. Mild encephalopathy was seen in two dogs. A third dog was not sacrificed but exposed as follows: 300 ppm, three times; 400 ppm, seven times; 500 ppm, two times; 600 ppm, one time; 800 ppm, three times; and 1000 ppm, one time. At each of these steps there was temporary leucopenia. After recovery of the white cell count, the concentration of CTFE was raised to the next level. An increase in the red blood cell count was detected during the 400 ppm exposure; plasma cholesterol rose slightly.

Following the exposure to 1000 ppm, the dog was subjected by Clayton, as reported by Hood (10) to the additional stress of exercise using a treadmill, the objective being an attempt to simulate a real-life problem where accidental exposure could be associated with considerable muscular exertion by the individual. Heart rate was the parameter chosen to assess this potential problem. Upon exercise, the dog became weak and was unable to complete a standard exercise pattern that his cohorts had no difficulty in completing. Heart rate was significantly elevated to 200 beats/min. No changes in cardiac rhythm were detected even though a high, endogenous epinephrine level was probably reached. After his exercise period, recovery to base line heart rate was slowed.

In order to segregate the effects of the parade of exposures which had preceded the final one at 1000 ppm, a fourth dog was exposed once, for 4 hr, to 1000 ppm. Effects on this animal were not nearly as dramatic. No acute toxic effects were reported, and there was a transient drop in the white cell count from 7100 to 2200 in the day following the exposure. Pathologic examination revealed degenerative neural changes which were not pronounced in the dog which had received multiple exposures than in the dog which had been exposed once to 1000 ppm.

Short-term, repeated exposures in dogs therefore cause transient leucopenia, an adaptive component (?), persistent erythrocytosis, a slight rise in plasma cholesterol, and encephalopathy without clinically detectable neurological abnormalities.

These findings strongly indicated that long-term, repeated exposures could ultimately damage the adaptive competence of the dog and provoke clinically observable neurological changes to accompany the encephalopathy. Accordingly, in Phase II, a long-term investigation was carried out by these workers. Rats, rabbits, guinea pigs, and dogs were exposed to CTFE concentrations beginning at 15 ppm (38 exposures) and raised progressively to 30 ppm (28 exposures), 50 ppm (93 exposures), 100

ppm (56 exposures), and 150 ppm (104 exposures). The exposures lasted 6 hr each day (excepting holidays) and were conducted 5 days per week for 14 months. Guinea pigs and rabbits were not affected, clinically or anatomically by the exposure regimen. Rats developed degenerative changes in the renal tubules which were discovered at the end of the 14 months of exposure. It is not known from the report of this work when or at what concentration of those named above the kidney response occurred. From Radford's data previously cited, one would expect the change to occur at the beginning of the 100–150 ppm exposure series and progressively worsen for the remainder of the 14 months.

In the group of dogs in this experiment, overt signs of toxicity were not noticeable through the exposures up to 150 ppm. At this level, neurological disturbances were observed in two dogs after 27 or 64 exposures. Changes in blood pressure became statistically significant shortly after the 100 ppm series was begun (Fig. 5). There was some hint of these changes near the finish of the 50 ppm series. It is probable that extending the 50 ppm level beyond the 93 exposures would have gradually increased the departure of the blood pressure parameters from control limits.

Hematological evidence of changes attributable to CTFE was also picked up during the 50 ppm series and became statistically significant at the higher levels. These responses were consistent with changes in hematologic measurements made during Phase II, viz., leucopenia, erythrocytosis (RBC, Hemoglobin, Hematocrit), granulocytopenia, and a slight elevation in plasma cholesterol in two dogs.

Pathologic examination of the dogs revealed muscle atrophy and degenerative changes in the brain, spinal cord, spinal and peripheral nerves. On the grounds of these data, the authors suggest that 20 ppm would be a safe "ceiling" concentration for workers potentially exposed 8 hr/day. Human data were evidently not available for entry into this judgement. Accidental human exposure has not been reported. Therefore the mode of toxic action of CTFE in humans is, at present, not known. Until this point is delineated, those who might be exposed to CTFE or supervise those who might, could, from the animal data just discussed, anticipate almost any organ system to be affected—respiratory, nervous, renal or hematopoietic.

The Russian investigator, Kochanov has published results of chronic animal toxicity experiments on CTFE (12). In this study, seven rabbits were exposed 4 hr daily except Sundays and holidays for 58 days to 500 ppm of CTFE. Three rabbits died during this sequence at days 16, 29, and 30. The concentration of CTFE was lowered on ex-

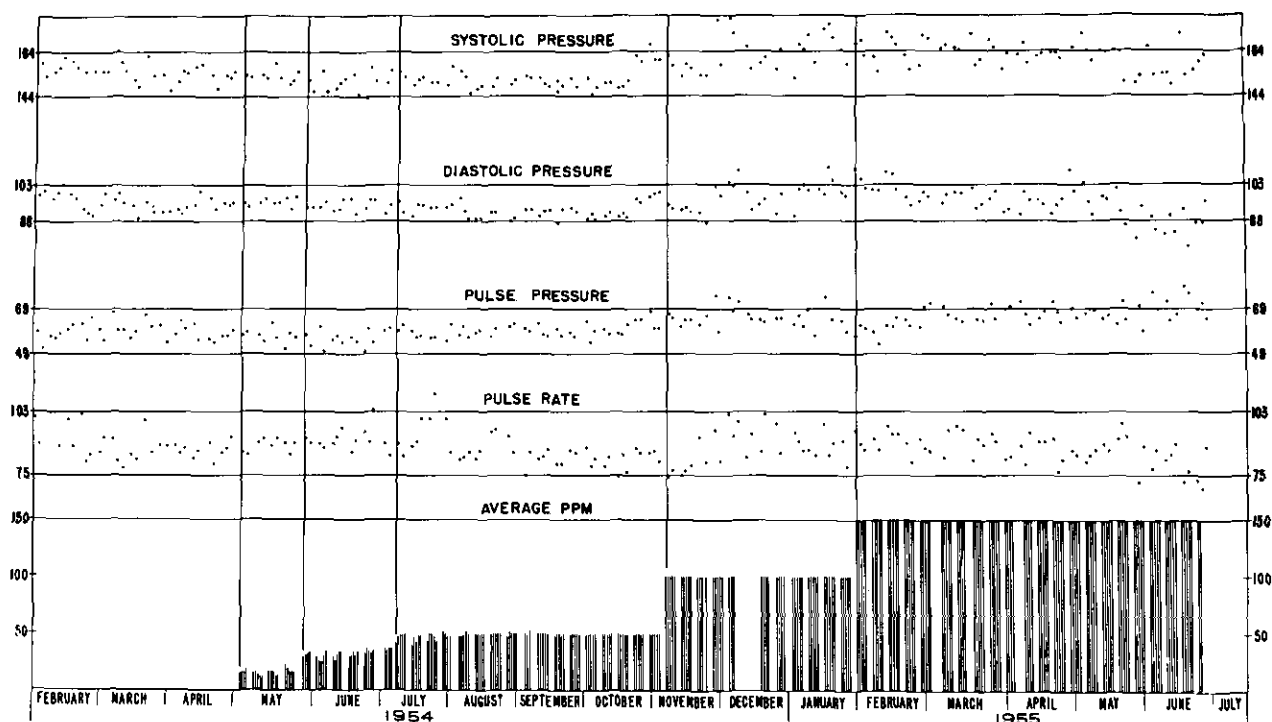


FIGURE 5. Vital signs in dog on long-term exposure to chlorotrifluoroethylene.

posure number 59 to 250 ppm with 6-hr exposures conducted. Rabbits experienced a reduction in motor activity and respiratory rate. Although varied, the CTFE group showed a decrease in rate of body weight gained as compared to the control group. Alkaline phosphatase values dropped steadily throughout the 81 to 130 day exposure period. Cholinesterase activity was increased 2 to 10 times by CTFE exposures. No significant differences in organ to body weight ratios were noted. Histopathologic examination revealed a widespread congestion of liver, spleen, and kidneys.

On comparing Kochanov's results (12) with those of Hood et al. (10), a noteworthy similarity emerges. Hood and co-workers exposed three rabbits at a lower concentration (300 ppm, Phase I and 50-150 ppm, Phase II). Neither of these proved injurious to the rabbits, although it is not given in their report whether or not they included some of the same clinical tests (alkaline phosphatase cholinesterase) employed by Kochanov. Hood et al. detected no depression in growth rate or pathologic change whereas, presumably because of the higher CTFE concentration, Kochanov did, and cites nonspecific congestive changes in some organs, but without a significant decline in body weight values.

A second chronic toxicity study (12) was undertaken by Kochanov, using rats and rabbits exposed

to 250 ppm CTFE, 6 hr/day over a period of 70 to 110 days. Only rabbits showed a decline in growth, as was true of the first series Kochanov conducted. Rat growth lagged only slightly behind that of controls. Rabbits displayed an increase in heart rate when held in an upright position and a slowed heart rate recovery period. Hematology of the rabbits disclosed a hypochromic anemia, reduction of the hemoglobin concentration, and number of erythrocytes; he also saw leucopenia. Hood et al. did not report hematologic changes in rabbits, but leucopenia was observed by them in dogs.

Kochanov's rats displayed a considerable reduction in their ability to integrate subliminal impulses, and oxygen consumption was increased.

What do these experiments of Hood et al. (10) and Kochanov (12) mean with regard to biologic activity and health hazards of the fluoroalkenes? Much! CTFE, for example, emerges as a compound of widespread biologic activity capable of provoking changes in the central nervous, respiratory, hematopoietic, and renal systems.

Perfluoroisobutylene (PFIB), Repeated Exposures

Clayton (5) has reported the repeated exposure toxicity of PFIB. This was a short experiment,

considering the extreme toxicity of this compound and thus the need to evaluate its toxicity. Rats were exposed to 0.1 ppm for 4 hr/day for 10 days. None of the four rats died. During the exposures the rats were occasionally restless and developed tachypnea. Two of the four rats showed moist rales and all appeared cyanotic occasionally. Some body weight losses occurred but not of sufficient magnitude to depress the growth curve. At the end of the 2-week exposure period, the rats were sacrificed. No histologic changes were attributable to PFIB.

As noted above (Tables 1 and 5), PFIB is the most toxic of the fluoroalkenes for which studies have been published so far. Because of its high order of acute inhalation toxicity, a safe industrial level is a most important question. Repeated exposures of rats lasting 10 days showed that 0.1 ppm PFIB was tolerated by rats but there was evidence of respiratory impairment. Therefore, before a threshold limit value (TLV) could be reasonably established for PFIB, repeated exposures of longer duration must be conducted. It is probable that under these exposure conditions, injury to organ systems distant from the lung could be affected, if PFIB acts as its companion chemicals, CTFE, HFP and TFE. Functional changes, preceding anatomic lesions, need to be described and related to the chemical properties of the individual fluoroalkenes.

Dichlorohexafluorobutene-2 (DCHF B) an Impurity in Halothane Anesthetic

The introduction of the fluorinated anesthetic, halothane (1,1,1-trifluorobromochloroethane, $F_3C-CHBrCl$) in the mid 1950's constituted a landmark in the field of anesthesiology. While it did not become the ideal anesthetic, it was far superior to the anesthetics then available. It is nonflammable and nonexplosive at anesthetic levels and as mixed with nitrous oxide and ether. It is also reported not to attack the metals and plastics with which it makes contact in vapor generators (17). Metabolically, halothane displayed the desirable qualities of rapid uptake, absorption by adipose tissues, and rapid elimination from the body. These features meant desirable anesthetic properties of rapid induction, ease of maintenance, and rapid recovery. Animal toxicity experiments disclosed a low order of inhalation toxicity for halothane and a minimal hepatotoxic action, (17).

In 1963, Cohen et al. isolated 2,3-dichloro-1,1,1,4,4,4-hexafluorobutene-2 (DCHF B) from halo-

thane and reported (18) that its concentration could increase tenfold in the vapor generator, Copper Kettle, employed to create anesthetic concentrations of halothane in clinical use. Conditions promoting the increase of DCHF B in halothane were enrichment due to the greater evaporation rate of halothane compared to DCHF B and the reaction of halothane with copper and oxygen which yielded DCHF B. Both *cis* and *trans* isomers were present. Because of the toxicity of fluoroalkenes, it then became highly important to study the toxicity of DCHF B especially because humans undergoing the stress of surgical procedures could prove susceptible to the toxic action of DCHF B.

Bunker and Blumenfeld (19) reported cases of human hepatic necrosis following administration of the anesthetic halothane, $CF_3CHBrCl$. In investigations of possible contaminants in halothane, Cohen et al. (18) observed that DCHF B was one of several minor contaminants in commercial samples of halothane anesthetic, then present in undiluted halothane at a level of 100 ppm by volume or at a concentration of 2 ppm by volume in gaseous anesthetic mixtures employing halothane. In 1963, Cohen et al. (18) reviewed the known toxicity of the butene, showing that it was acutely toxic for a dog in anesthetic concentrations. Delayed onset of anesthesia was observed and this was followed by convulsions and death in 1 hr. About the same time, another study by Chenoweth (20) showed that DCHF B was lethal for rats in a 4-hr exposure at 100 ppm, but that some rats survived 100 ppm exposure of 1 or 2 hr duration. Response of rats in the lethal exposures was consistent with pulmonary irritation. Pulmonary congestion and edema were observed post-mortem. Degenerative changes in the kidney and liver were also observed. In another study, this author also observed fatalities in rats from severe injury to lungs, liver, and kidney on inhaling this fluoroalkene for 4 hr at 5 ppm.

In experiments conducted by Cohen et al. in 1965 (21), 85 male rats were exposed to $CF_3-CCl=CCl-CF_3$ in a dynamic system for 3-hr periods. Results indicated an LD_{50} for this exposure of 52 ppm, with 100% fatalities at 105 ppm. Respiratory deaths occurred in 6 to 24 hr at 840 ppm, and delayed deaths in 4 to 14 days resulted from exposures as low as 52 ppm. The latter group lost weight, slowly becoming lethargic, and expired. The rats exposed to the higher concentrations of DCHF B showed acute hemorrhagic changes in the lungs, while the groups exposed to 105-210 ppm showed a healing interstitial pneumonitis and central lobular necrosis of the liver.

Studies of the effects of $CF_3-CCl=CCl-CF_3$

on dogs by Cohen et al. (21) included induction of anesthesia with thiopental (150–200 mg), followed by a 3-hr inhalation of 1% halothane with added increments of the butene. Vaporization of the anesthetic was with 100% oxygen. Following insertion of the endotracheal tube, ventilation was supported with a respirator. Electrocardiogram, arterial blood pressure, and the electroencephalogram were monitored and intermittent determinations of pH, P_{O_2} , and P_{CO_2} were made. Initially, control animals were paired with the experimental group, and the former maintained on 1% halothane without added DCHFB. The LD_{50} determined in the dog was 200 ppm in 1% halothane for a 3-hr exposure, decreasing to 150 ppm for a similar anesthesia repeated on four consecutive days.

At 100 ppm, recovery from anesthesia followed a normal course. Clinically, at high levels of DCHFB, the electroencephalogram revealed frequent episodes of convulsions. Analysis of blood levels of DCHFB during seizures disclosed a level of 31 $\mu\text{g}/100\text{ ml}$ of blood. Halothane anesthesia evidently protected the dogs from violent convulsions, because the latter appeared about 15 min after anesthesia was ended and halothane levels in the body dropped, while, probably, DCHFB levels remained sufficiently high. The seizures, if not controlled by barbiturates and diphenylhydantoin, were fatal in 6–8 hr. A DCHFB concentration of 200 ppm provoked hyperirritability, not convulsions. Death in 7 to 10 days was preceded by a downhill clinical course—appetite loss, weight loss, and lethargy. Post-mortem examinations showed only occasional renal tubular damage.

In an exposure system similar to that used for dogs, Cohen found the LD_{50} for the rhesus monkey, calculated by the method of maximum likelihood, to be 54 ppm for a 3-hr exposure. Death occurred within 4 to 9 days. Convulsive disorders appeared in the two animals exposed to 350 and 400 ppm. Pathology revealed severe pulmonary changes in all animals. Grossly, areas of pneumonic consolidation were present, and microscopically the alveolar walls were edematous with occasional areas of necrosis and covered with a thick exudative layer. Animals living past the fifth day evidenced liver damage, the extent of which appeared related to the concentration of DCHFB. At the lowest fatal concentration the liver showed atrophic changes and an increase in lipid content, while at the highest concentration the animal showed central lobular necrosis of the liver.

Needless to say, the discovery of an impurity which displayed high toxicity and constituted a hazard to the lives of surgical patients was of extraordinary consequence. Raventos and Lemon

(22) reviewed the manufacturer's purity standards. In 1956, contaminant specifications called for no more than 0.1% by weight of total volatiles and no more than 0.05% v/v for any one impurity. In 1965 the same specifications were 0.05% v/v and 0.01% v/v, respectively. Analyses of halothane by these authors revealed a *cis-trans* composition of 1:6. Their toxicity studies included these isomers, isolated by preparative gas-liquid chromatography, as well as the mixed isomers as appearing in regular manufacture.

Raventos and Lemon (22) investigated in mice, rats, rabbits, monkeys, and dogs the acute inhalation toxicity of DCHFB, other fluoroalkenes, and fluoroalkanes which have been found as trace contaminants in halothane as manufactured. These authors confirmed the high order of toxicity of DCHFB [4-hr LC_{50} : rats, 16 ppm; mice, 26 ppm; dogs, 182 ppm; monkeys (3-hr), 90 ppm]. Table 6 presents the data on acute inhalation toxicity reported by Raventos and Lemon (22) for DCHFB. On the basis of these data, the order of toxicity for the four species studied is rats > mice > monkeys > dogs. Compared with PFIB, DCHFB is an order of magnitude more toxic, based on rat data.

Table 6. Species variation and relation to time of exposure for acute inhalation toxicity of dichlorohexafluorobutene-2.

Species	LC_{50} , ppm				
	1 hr exposure	2 hr exposure	3 hr exposure	4 hr exposure	6 hr exposure
Mice	55	39	—	26	20
Rats	47	28	—	16	—
Dogs	725	415	—	182	115
Monkeys	186	139	90	—	—

^aData from Raventos and Lemon (22).

In spite of these differences in quantitative toxicity of DCHFB, the pathological changes in the lungs were quite uniform among the various species studied. The pulmonary reaction was evident as dyspnea and cyanosis during or after exposure. Histology disclosed only pulmonary congestion and edema. Unlike the report of hepatotoxicity of DCHFB by Cohen (21), Raventos and Lemon (22) observed no significant liver change; renal lesions (small thromboses in glomerular capillaries, vacuolation and degenerative changes in the first part of the proximal tubules, and increase in the number of colloid casts) were observed in only a few rats inhaling 330–550 ppm for one hour. Raventos and Lemon also found that the *trans* isomer of DCHFB was approximately three times as toxic for mice as the *cis* isomer (1-hr LC_{50} : *trans*, 61 ppm; *cis*, 179 ppm);

however, the 1-hr LC_{50} for the *trans* isomer was not significantly different from that of *cis-trans* mixture (LC_{50} , 55 ppm).

Cohen et al. studied metabolism and uptake of DCHFB (21). Analyses of outflow blood from the liver of two monkeys inhaling 1500 ppm DCHFB for 2 hr revealed a lower amount of DCHFB than inflow blood showed. Evidence was obtained that DCHFB was altered by the liver cells, because DCHFB *per se* could not be identified with electron capture chromatography. The uptake studies on man indicated that DCHFB passes through the alveolar wall and apparently is metabolized by the subjects because exhaled DCHFB concentrations were lower than those inhaled, and DCHFB did not build up in the vaporizer which was supplying 1% vol/vol halothane.

Other fluoroalkene contaminants of halothane were studied in a preliminary manner by Raventos and Lemon (22). The 1-hr LC_{50} of $CF=CClBr$ for mice was 250 ppm. In addition to lung damage this compound also produced kidney changes. This is in agreement with the kidney effects reported above for chlorotrifluoroethylene, tetrafluoroethylene, and hexafluoropropylene. For the *trans* isomer of $CF_3-CH=CBr-CF_3$ (BHFB), the 1-hr LC_{50} for mice was 5000 ppm. Lung changes, congestion, and edema were the predominant pathological changes, but there was some increase in the number of liver cells staining for lipids, however, without degenerative changes. Exposures to the *trans* isomer of the chloro analog, $CF_3-CH=CCl-CF_3$ (CHFB) at 16,000 ppm were not lethal for mice. Convulsions were observed at 5000 ppm and higher. The histological changes were similar to those observed with $CF_3-CH=CBr-CF_3$.

Ferstandig (23) has published a preliminary note on the inhalation toxicity of a 50:50 mixture of *cis-trans* isomers of CHFB. For rats exposed for 15 min, the LC_{50} of this mixture was 78 ppm, equivalent to approximately 20 ppm for 1-hr exposure. Unless there is a marked difference in the toxicity of this compound for rats as compared to mice, which were used by Raventos and Lemon (22), it would appear that the *cis* isomer is many times more toxic than the *trans* isomer as noted above. This is not the case for DCHFB, for which Raventos and Lemon found the *trans* isomer about three times as toxic as the *cis* isomer. The reports of Cohen et al. (21) and Raventos and Lemon (22) are contradictory, in that the former reported hepatotoxicity for DCHFB, while Raventos and Lemon observed no such change. Also, there are evidently significant differences in toxicity between the *cis* and *trans* isomers of $CF_3-CCl=CCl-CF_3$ and $CF_3-CH=CCl-CF_3$. These questions need

clarification. On balance, it appears that these fluoroalkenes have a relatively low hepatotoxic potential and, as such, would seem not to contribute significantly to the post-operative hepatic disease reported above in rare cases following halothane anesthesia.

A study reported by Clayton (15) of the acute inhalation toxicity of a related butene, $CF_3-CF=CH-CF_3$, showed that 200 ppm was the approximate lethal concentration (ALC) for a 4-hr exposure of rats. Observations indicated that the compound acts predominantly on the central nervous or respiratory systems. Rats became sedated during exposure; after exposure, there were weight losses, polypnea, and quivering. Death occurred in 2-3 days. Necropsy was not conducted.

On comparing the two butenes cited above, namely, $CF_3CH=CClCF_3$ and $CF_3CH=CFCF_3$, it is noted that the chlorinated compound appears to be much more toxic than the fluorinated compound. Effects on the liver were reported in rats for the former. There are no comparable data on the fluorinated compound. It is notable that single and repeated inhalation studies on CTFE by Hood (10) did not reveal liver damage in rats, rabbits, guinea pigs, or dogs. Therefore the fluoroalkenes cannot be lumped together when considering their toxic actions and biologic mechanisms.

Health Effects of Fluoroalkenes

Ferstandig (23) reported an accidental exposure of two humans who were handling CHFB. It is estimated that the two people were exposed about 1 hr to less than 10 ppm. They experienced drowsiness and shortness of breath 4-6 hr after exposure. Pulmonary fibrosis developed in 2-3 days but later resolved. It is evident that this material should be handled with considerable caution in this class of fluorocarbons.

In *L'Express* of September 25, 1967, the tragic events following the exposure of 14 Pechiney employees to dichlorohexafluorobutene were reported (24). This fluoroalkene was a primary material for the manufacture of a herbicide. Three of the workers exposed died from pulmonary edema and fibrosis within about two weeks after the accidental exposure. Oxygen, cortisone, and antibiotics were not successful in treating these three of the fourteen men exposed. It is not clear how the exposure occurred. In the same article, Maurel refers to a fatal exposure in 1964 when the compound was being studied as a degreasing agent. A laboratory technician succumbed evidently from exposure in a research laboratory.

In accidental exposures to fluoroalkenes occurring in the manufacture of fluorocarbons, the events are difficult to evaluate because several compounds may be involved, and thus the sequelae of such exposures are not readily assignable to any one material. It would be expected that humans inadvertently inhaling fluoroalkenes would suffer pulmonary impairment or injury, depending on the concentration and duration of the inhalation. Upper respiratory irritation is likely not to occur and therefore personnel at risk would have no sensory warning to avoid exposure.

At exposure levels sufficient to produce pulmonary injury, this would dominate the clinical response. Other organ systems might not be affected. However at levels low enough not to cause fatal pulmonary impairment, the possibility of effects on other systems becomes an important consideration as to human health hazards. The models illustrating this likelihood are the long-term animal studies on chlorotrifluoroethylene by Hood et al. (10) and Zapp (8).

It is clear that precautionary measures should be taken to obviate the hazards inherent in this class of fluorocarbons.

Exposure of the general public to fluoroalkenes does not seem to be a significant probability, because these fluorocarbons do not now occupy the marketplace in significant quantities. Should an increase in market penetration of the fluoroalkenes eventuate, much care will have to be exerted to avoid unwanted exposures and additional toxicologic studies will be required to document more fully the biologic activity of this class of fluorocarbons.

Research Needs

Cook and Pierce (3) have alluded to the nucleophilic sensitivity of the fluoroalkenes and have ascribed the relative toxicities of PFIB, HFP, and TFE to this attribute. They further compared the fluoroalkenes to highly active alkylating agents which are also characterized by sensitivity to nucleophiles, an abundance of which are present in biologic loci. Because of this similarity, definitive studies are needed to evaluate the possibility that fluoroalkenes, when contacting biologic systems, act as alkylating agents. This brings up questions regarding potential carcinogenic, mutagenic, and teratogenic effects of the fluoroalkenes.

Truhaut et al. (25) have taken a different approach to the toxic action of fluoroalkenes, especially DCHFB. In animal experiments, using an

intratracheal exposure with 500 ppm and 2000 ppm DCHB, they noted a strong acidosis. They conclude that formation of trifluoroacetic acid could account for the pulmonary damage they observed in rats. Lacking data, this potential mechanism needs exploration.

Danishevskii and Kochanov (4) have suggested that the reason for the high inhalation toxicity for perfluoroisobutylene is the formation of carbonyl fluoride, COF_2 , in the lung. No experimental data are presented to establish this possibility. In fact, COF_2 is basically HF toxicity as shown by rat exposures described earlier and PFIB is considerably more toxic than COF_2 . Thus the suggestion of Danishevskii and Kochanov would appear unlikely.

Further research on the toxicity of the fluoroalkenes is needed in the following areas.

(1) Long-term, repeated exposures using at least two species three levels + two controls (1 comparative) should be made to establish TLV, assess progressive functional and structural kidney changes, assess carcinogenic potential, with serial sacrifice and recovery studies.

(2) Effects on rat reproduction with three generations, two litters per generation, with studies of kidney and liver functions at F_1b , F_2b , and F_3b , histology at F_2b and F_3b , two or three test levels and appropriate controls, and reproductive indices, including studies in which exposures are begun at weanling F_0 .

(3) Teratology studies of rabbit and one other species require three test levels and one control, and should include behavioral studies.

(4) Mutagenesis studies should be performed on mammalian and nonmammalian systems.

(5) Metabolic studies should include studies of lung enzymes and induction processes, alveolar macrophage response (phagocytosis, enzyme profiles, viability, O_2 uptake, and other measurements, e.g., column separation), systemic distribution, namely absorption (tagged compounds) and excretion (fluoride, organic fluorocarbons), organ perfusion, especially kidney, effects on kidney tubules by electron microscopy, scanning and transmission, and blood levels.

(6) *In vitro* toxicology studies on human cells should be performed.

(7) Pathology studies should include light and electron microscopy studies.

(8) Physiology of upper respiratory tract changes and mechanical, ventilatory, and diffusion properties of lungs should be investigated.

(9) Effects on several mammalian species, similar to the work of Hood (10) should be performed.

(10) Epidemiologic studies of occupational populations are needed.

(11) Carcinogenic studies should include investigation of cell transformation and carcinogenicity in long-term, low-level exposures.

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